510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k050029

B. Purpose for Submission:

New Device

C. Measurand:

Myeloperoxidase

D. Type of Test:

Quantitative

E. Applicant:

Prognostix, Inc.

F. Proprietary and Established Names:
CardioMPO Reagent Kit
CardioMPO Calibrator Kit
CardioMPO Control Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5600
   21 CFR 862.1150
   21 CFR 862.1660

2. Classification:
   Class II
   Class II
   Class I

3. Product code:
   NTV (Myeloperoxidase, Immunoassay, System, Test)
   JIS (Calibrator, Primary)
   JJX (Quality Control Material)

4. Panel:
   82 (Immunology)
   75 (Clinical Chemistry)

H. Intended Use:

1. Intended use(s):

   See Indications for Use statement below.

2. Indication(s) for use:

   The *Cardio*MPO™ Test is comprised of the *Cardio*MPO Reagent Kit, the *Cardio*MPO Calibrator Kit, and the *Cardio*MPO Control Kit.

   The *Cardio*MPO Reagent Kit is an enzyme immunoassay intended for the quantitative determination of myeloperoxidase in human plasma, to be used in conjunction with clinical history, ECG and cardiac biomarkers to evaluate patients presenting with chest pain that are at risk for major adverse cardiac events, including myocardial infarction, need for revascularization, or death.

   The PrognostiX *Cardio*MPO Calibrator Kit is intended for use with the *Cardio*MPO Reagent Kit to establish a calibration curve that is used to determine MPO concentration.

   The PrognostiX *Cardio*MPO Control Kit is intended for use with the *Cardio*MPO Reagent Kit as an assayed quality control sample to monitor and evaluate the precision and accuracy of the *Cardio*MPO Test.

3. Special conditions for use statement(s):

   For Prescription Use

4. Special instrument requirements:

   Microtiter plate reader capable of reading at 450 nm

I. Device Description:

The Prognostix CardioMPO Enzyme immunoassay reagent kit contains one mouse monoclonal antibody specific to myeloperoxidase (MPO), assay buffer, primary rabbit polyclonal anti-MPO antibody, secondary goat anti-rabbit IgG antibody conjugate, substrate reagent, stopping solution, wash buffer concentrate and plate sealer. The calibrators and controls are sold separately.

The Prognostix CardioMPO Calibrator kit contains six concentrations of human MPO

in phosphate buffered matrix. The calibrator kit is provided in a ready to use frozen liquid form.

The Prognostix CardioMPO control kit contains three concentrations of human MPO in human lithium heparin plasma. The control kit is provided in a ready to use frozen liquid form. The human source materials in the Prognostix CardioMPO control kit was tested and confirmed negative for HIV 1 and 2, HBV and HCV with FDA approved or licensed assays.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   diaDexus PLAC™ Test

2. Predicate 510(k) number(s):

   k030477

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Methodology	ELISA	ELISA
Detection Method	Optical Density at 450 nm	Optical Density at 450 nm

Differences		
Item	Device	Predicate
Intended Use	Quantitative determination of myeloperoxidase in human plasma, to be used in conjunction with clinical history, ECG and cardiac biomarkers to evaluate patients presenting with chest pain that are at risk for major adverse cardiac events, including myocardial infarction, need for revascularization, or death	Quantitative in vitro diagnostic test for the determination of Lp-PLA2 in human plasma, to be used in conjunction with clinical evaluation and patient risk assessment as an aid in predicting risk for coronary heart disease.
Analyte	Myeloperoxidase	Lipoprotein-associated phospholipase A2
Sample	Lithium Heparin Plasma	EDTA Plasma

K. Standard/Guidance Document Referenced (if applicable):

NCCLS Document EP5-A: Evaluation of precision performance of clinical chemistry devices.

NCCLS Document EP6-A: Evaluation of the linearity of quantitative measurement procedures; a statistical approach.

NCCLS Document EP7-A: Interference testing in clinical chemistry.

L. Test Principle:

Sandwich enzyme immunoassay using two specific antibodies, one monoclonal and one polyclonal

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was conducted based on NCCLS EP5-A. Four matrix controls and five plasma controls were run in duplicates, twice a day for 20 days (N=80). Matrix controls were comprised of human MPO derived from cell culture in a phosphate-buffered matrix. Plasma controls were comprised of human MPO derived from cell culture in human lithium heparin plasma. For matrix control 1 and 3 and plasma control 1, N=76 (19 days) due to outliers caused by pipetting errors and control concentrations. The results are summarized below.

Sample name	Test MPO concentration (pM)	Number of replicates	Total %CV	Within-run %CV
Matrix Control 1	2602	76*	5.8%	4.7%
Matrix Control 2	1240	80	7.2%	6.6%
Matrix Control 3	605	76*	7.1%	6.7%
Matrix Control 4	198	80	10.9%	6.5%
Average			7.8%	6.1%
Plasma Control 1	440	76*	9.5%	4.6%
Plasma Control 2	586	80	8.3%	6.3%
Plasma Control 3	562	80	9.4%	5.8%
Plasma Control 4	944	80	6.1%	5.1%
Plasma Control 5	1310	80	7.9%	5.6%
Average			8.2%	5.5%

The average total % CV for the matrix controls and plasma controls were 7.8% and 8.2% respectively. The average within-run % CV for the matrix controls and plasma controls were 6.1% and 5.5% respectively.

Precision was conducted to determine the variability of the Prognostic CardioMPO Test using different lots of the reagent kit. Three lots of the CardioMPO reagent kits were analyzed with nine patient samples, four matrix controls and five plasma controls. Between-lot variability was analyzed by comparing the mean MPO concentration of each sample measured using one reagent kit lot against the overall MPO concentration of each sample across all three reagent kit lots using Passing and Bablok regression. The linear equations for the three lots were Y=1.0023X -50.171, Y=0.9971X +22.759 and Y=1.0249X + 14.168 respectively. The sponsor concludes that no significant difference in MPO measurement related to different CardioMPO reagent kit lots.

b. Linearity/assay reportable range:

Linearity was conducted according to NCCLS EP6-A. Five high MPO plasma samples were prepared by spiking 5 plasma samples to reach a MPO concentration above the expected linear range. Four low MPO plasma samples were created by mixing the previously prepared high MPO samples with neat plasma samples. Five test samples were analyzed in triplicates with Prognostix CardioMPO reagent kit. Linearity was assessed using the polynomial evaluation of linearity, whereas the high and low level concentrations at which all three non-linear coefficients were found not to be significant (within a 95% confidence interval) were considered the linear range for that sample. A linear regression was completed and the percent difference between the apparent MPO concentration at each test level and the MPO concentration predicted from the linear regression was calculated. The five sample results were compiled and the greatest MPO concentration that remained within the linear range was 5223 pM and the lowest was 10 pM. Please see the chart below.

Results of the linearity evaluation of five Test Samples
(Shaded values were found to be outside the linear range)

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
Level	Mean MPO conc. (pM)	% difference from regression	Mean MPO conc. (pM)	% difference from regression	Mean MPO conc. (pM)	% difference from regression	Mean MPO conc. (pM)	% difference from regression	Mean MPO conc. (pM)	% difference from regression
1	268	-0.4	478	0.0	408	-14.0	108	1.0	10	80.4
2	894	4.0	1475	0.0	948	0.2	742	-0.1	597	2.3
3	1599	-0.1	2501	-1.1	1633	-5.9			1183	-1.1
4	2261	0.0	3636	-4.7	1903	-0.2	1848	7.8	1791	-3.5
5	2903	0.8	4469	0.0	2726	0.0	2637	0.0	2346	-2.4
6	3800	-5.8	5223	4.5	3600	-8.5	3391	-3.8	2815	1.3
7	4348	-2.1	5847	9.6	3813	2.5			3461	-1.5
8	4871	1.0	6456	100.0	4497	0.1			3833	3.5
9	5340	4.4	6299	100.0	5180	-1.7	4815	6.7	4590	-1.3

The linear equations for the 5 samples were

Sample 1 Y= 665.03X-398.18,

Sample 2 Y= 997.9X-520.36,

Sample 3 Y=592.11X-234.3,

Sample 4 Y=631.73X-522.84 and

Sample 5 Y=566.15X-515.08.

The sponsor states that samples at the low end of the linear range were somewhat imprecise when compared to the rest of the range and that the imprecision was within 60 pM. The sponsor also stated that 60 pM is not clinically relevant at low MPO concentrations. The remaining samples revealed less than 9% deviation from the linear regression result. The CardioMPO test is linear from 13 to 5223 pM MPO, within 60 pM or 9% in this interval.

The hook effect was studied to determine if plasma samples containing excessively high levels of MPO can cause a hook in the absorbance that would result in reportable MPO reading when no absorbance reading should be available. Purified MPO was diluted in calibrator matrix concentrations up to 800,000 pM MPO, which is approximately 150 times greater that the upper end of the reportable range and 500 times greater that the median levels of patients in the clinical study. All the samples were reported as having concentrations greater than the reportable range.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The CardioMPO controls and calibrators are prepared from commercially available concentrated human MPO stock. MPO from the lysed cells is purified. An intermediate stock solution is prepared gravimetrically from the MPO stock solution and calibrator matrix. A working MPO stock solution is prepared by further diluting the intermediate stock solution in calibrator matrix. Calibrators A – E are prepared gravimetrically adding working stock solutions to calibrator matrix. Calibrator F is comprised of calibrator matrix only. Controls 1-3 are prepared by gravimetrically adding the working MPO stock solution to human lithium heparin plasma.

Values are assigned to new calibrators through the use of a set of six gold standard calibrators. Gold standard calibrators were created from a single lot of MPO. New working calibrator lots are analyzed against the gold standard calibrators in the CardioMPO test in duplicate for five days at two runs per day. The mean MPO concentrations calculated from the twenty replicates are

verified for precision and concentrations are assigned to the new working calibrators. Concentrations values and ranges are assigned for new controls lots though the same assignment process as for the calibrators, although the new controls may be analyzed against working calibrators.

Stability

Two accelerated stability studies were conducted with the CardioMPO Control kit- Opened/thawed and Unopened/frozen. Plasma controls with MPO concentrations spanning the calibration range (one neat plasma sample and two or three plasma samples spiked with MPO) were used to simulate stability of both controls and plasma samples and stored at 45°, 35°, room temperature and 4°C, removed and assayed periodically. The sponsor suggests storing plasma samples and controls for no more than 8 hours at room temperature (20-26°C) and 14 days at 4°C. The results from the accelerated stability studies at elevated temperatures claim the controls/plasma samples will be stable for greater than one year at -20°C.

To evaluate freeze thaw stability conditions, the same plasma controls were stored at -20°C and -80°C, thawed, assayed and returned to frozen storage. No degradation of MPO was observed over 2 freeze/thaw cycles for 18 days at -20°C or 7 freeze/that cycles for 21 days at -80°C.

To evaluate unopened/frozen controls, the plasma controls discussed in the open/thaw study above were stored at -80°C. Periodically a set of controls were removed, thawed and assayed with the CardioMPO test. No MPO degradation was observed in unopened, frozen controls at 41 days.

Real-time controls/plasma sample stability studies are ongoing; the control kit will be labeled as stable for 6 months.

Two accelerated stability studies were conducted with the CardioMPO Calibrator kit- Opened/thawed and Unopened/frozen. Calibrators were stored at 45°, 35° and 4°C and were periodically removed from storage, assayed and returned. At 45° and 35°C, the calibrators lost ~37% and ~23% after one day respectively. When stored at 4°C, the calibrators maintained 99% of their initial MPO concentration after 14 days and 86% after 43 days. The sponsor recommends storing calibrators for no more than 14 days at 4°c.

To evaluate unopened/frozen calibrators, several set of calibrators were aliquotted and stored at -80°C. Periodically, a set of calibrators was removed, thawed and assayed in the CardioMPO test. No MPO degradation was observed in unopened, frozen calibrators after 41 days. The sponsor states that unopened vials will be labeled as stable for 6 months.

d. Detection limit:

The zero calibrator material (F) was assayed 24 times. The mean plus two standard deviation value was calculated by interpolation as 13 pM myeloperoxidase.

e. Analytical specificity:

Three studies were conducted to assess analytical specificity- cross reactivity, interfering substances and interfering antibodies and were assessed according to NCCLS EP7-A.

Cross reactivity was conducted on eleven potentially cross-reactive proteins at 3 difference concentrations in human plasma and assay buffer. Cross reactivity was calculated by the following equation:

$$\%\,\text{Cross-reactivity} = 100 \times \left(\frac{\text{Measured MPO conc. (pM) - True MPO conc. (pM)}}{\text{Cross-reactant concentration (pM)}} \right)$$

All controls were run (excluding the PBS blank) twice in duplicate and the cross reactivity samples were run in triplicate. All proteins showed less than 0.05% cross reactivity at the highest test concentration and less than 1.8% cross reactivity at the lowest test concentration when added to a plasma pool.

When added to the assay buffer, the proteins showed less than 0.09% cross reactivity at the highest test concentration and less than 0.2% at the lowest concentration. Elastase produced some slight cross reactivity but the sponsor determined it to be clinically insignificant. The results are shown in the table below.

Substance tested	Percent cross-reactivity					
	Interferent level in plasma			Interferent level in buffer		
	High	Mid	Low	High	Mid	Low
α 1 antitrypsin, human	-0.0132	-0.0228	-0.5008	-0.0010	-0.0037	-0.0725
C-reactive protein, human	-0.0264	-0.0459	-1.7441	-0.0014	-0.0066	-0.2090
Lysozyme, human	-0.0021	-0.0091	-0.0242	0.0000	-0.0015	-0.0070
IgA, human	-0.0263	0.0492	-0.3396	-0.0032	-0.0134	-0.1835
Elastase, human	0.0433	0.0090	-0.1060	0.0868	0.0435	0.0032
Lactoperoxidase, bovine	-0.0166	0.0036	-0.1117	-0.0018	-0.0076	-0.0818
Lactoferrin, human	-0.0111	-0.0408	-0.6815	0.0129	0.0163	-0.0879
COX 1, ovine	-0.0217	0.0309	0.5054	0.0006	-0.0009	-0.0090
COX 2, human	0.0031	0.0432	0.5584	0.0002	-0.0046	0.0453
Thyroid peroxidase, human	0.0066	0.1481	1.2454	0.0016	0.2529	0.0130
Troponin I, human	0.0042	0.0470	0.3948	-0.0003	0.0005	-0.0220

An interfering substances study was conducted to determine if substances likely to be in patient samples would interfere with the CardioMPO test.

Interference testing was conducted according to NCCLS EP7-A. 26 samples were spiked with MPO values of 1000 pM and 3000 pM. 16 drugs and dietary substances, 8 endogenous (which included triglycerides, hemoglobin and bilirubin) and 2 anticoagulants/preservatives were run in 8 replicates. An interference screen was conducted on 22/26 samples. A dose response study was conducted of hemoglobin, conjugated bilirubin (2) and triglycerides. 5 test pools were created by diluting the 3000 pM MPO plasma pool with high concentrations of interferent with a 3000 pM MPO plasma pool containing a low concentration of interferents. Percent interference was calculated by

$$100\times\left(\frac{\text{Mean MPO conc.(pM) of inter. substance} - \text{Mean MPO conc.(pM) of control pool}}{\text{Mean MPO conc. of control pool}}\right)$$

The sponsor's acceptance criterion of bias was interference greater than 10%. All substances listed, excluding hemoglobin and conjugated bilirubin did not cause interference with the CardioMPO test. The results of the plasma interference are listed in the chart below.

Interferent	Actual test concentration (mg/L)	Comments on test concentration
Drugs & dietary substances		
acetylsalicylic acid	600	Toxic dose
amoxicillin	75	3x therapeutic dose
captopril	5	Toxic dose
fenofibrate	45	3x therapeutic dose
hydrochlorothiazide	6	3x therapeutic dose
hydrocortisone	0.69	Toxic dose
ibuprofen	500	Toxic dose
indomethacin	36	2x therapeutic dose
isosorbide dinitrate	0.15	3x therapeutic dose
lovastatin	53	2x therapeutic dose
methotrexate	160	Therapeutic dose
metoprolol	5	Toxic dose
naproxen	500	4x therapeutic dose
niacin	0.5	5x therapeutic dose
nifedipine	0.4	2x therapeutic dose
salicylic acid	600	Toxic dose
Endogenous substances		
albumin, human	50000	*High
bilirubin, conjugated	50	*High
bilirubin, unconjugated	150	*High
ceruloplasmin	600	High
cholesterol	1000	*High
DNA	16.7	High
hemoglobin	5000	Gross hemolysis
triglycerides	5000	Gross lipemia
Anticoagulants & preservatives		
heparin, lithium	3000 U/L	3x therapeutic dose
sodium azide	0.50%	5x preservative dose

Hemoglobin, conjugated bilirubin and triglycerides were studied further in a dose response study. No interference was detected for triglycerides levels up to 5000 mg/L and for conjugated bilirubin levels up to 50 mg/L. The linear regression for triglycerides and conjugated bilirubin were Y= -0.0119X +2760 (R2=0.1231) and Y=0.0017X + 2710.4 (R2= 0.0038), respectively. Hemoglobin revealed slight interference when tested at concentrations greater than 1500 mg/L and none at values less than 1500 mg/L. Hemoglobin revealed a linear regression equation of Y= 0.0723X + 2834.5.

Heterophilic antibodies which included anti MPO p-anti-neutrophil cytoplasmic antibody (pANCA), HAMA, and RF were tested for interference with the Prognostix CardioMPO test. 3 groups of samples were analyzed.

Group 1 contained eight samples known to have elevated levels of HAMA or RF antibodies. Group 2 contained eight samples known to have elevated levels of heterophilic antibodies other than HAMA and RF (i.e. anti-goat & anti-rabbit). Group 3 contained five samples with elevated levels of p-ANCA specific to MPO (anti-MPO pANCA positive) were analyzed. Each plasma sample was diluted 1:1 in normal donor plasma and the heterophilic antibody sample and was analyzed for linearity. Interference for heterophilic antibodies was determined to be % recovery of greater than 20%. The percent recovery was calculated by dividing the measured MPO concentration by the expected MPO concentration. The HAMA/RF positive, HAMA/RF negative and antiMPO pANCA positive results showed a mean recovery of 111% (101-118), 107% (89-118) and 107% (101-114) respectively.

A spiking recovery study was conducted on 14 spiked plasma samples to determine the CardioMPO test ability to recover 3 levels of MPO (516 pM, 1016 pM and 1500 pM). The samples were analyzed in duplicates and the % recovery was calculated. The percent recovery equation was calculated by dividing the sum of the mean measured MPO concentration of the spiked sample by the sum of the mean measured MPO concentration of the neat plasma sample plus the MPO concentration of the spike. The average recovery for the 510 pM spike was 95.3% (range from 84.7% to 103.4%). The average recovery for the 1016 pM was 99.1% (range from 80% to 122.4%). The average recovery for the 1500 pM pike was 95.4% (range from 81.2% to 104.3%).

A dilution recovery study was conducted to determine the test ability to measure MPO in pre-diluted plasma samples (with assay buffer). 13 plasma samples were spiked with 1500 pM MPO and diluted to 4 levels (1:2, 1:4, 1:8 and 1:16) with assay buffer and analyzed for recovery by the CardioMPO test. The % recovery was calculated by dividing the mean measured MPO concentration of the pre-diluted sample multiplied by the dilution factor by the mean MPO concentration of the sample with pre-dilution. The average recovery of a 1:2 dilution was 116% (range from 102% to 131%), 1:4 dilution was 132% (range from 117% to 169%), 1:8 dilution was 140% (range from 113 to 175%) and 1:16 dilution was 144% (range from 115% to 167%). The sponsor states the test kit is formulated to account for matrix effects associated with a 5 μL human plasma sample. The sponsor does not recommend performing a sample dilution and that if a dilution is required; a 1:2 dilution is recommended with the knowledge that the MPO concentration obtained will be ~16% greater than the actual value.

f. Assay cut-off:

N/A

2. Comparison studies:

   a. *Method comparison with predicate device:*

      See results of the clinical study.

   b. *Matrix comparison:*

      N /A

3. Clinical studies:

   Two clinical studies were performed using the CardioMPO test; one myeloperoxidase in normal subjects that is presented in the expected values section of this template below and the second study was a re-analysis of myeloperoxidase in patients presented to an emergency room complaining of chest pain. The sponsor conducted a re-analysis of samples from a study published in the New England Journal of Medicine. The study examined the prognostic value of MPO levels in subjects with chest pain by using "research use only" MPO test reagents.
   The MPO levels in 560 banked lithium heparin plasma samples from the study were measured to determine the efficacy of the test as a predictor of risk for major adverse cardiac events (MACE). MPO levels in patients, 23 to 96 years of age, were measured using the CardioMPO test, calibrators and controls. The patients' had enrolled in an earlier study of the diagnosis of myocardial infarction with necrotic biomarkers. Some patients were excluded statistically if one of the following variables were missing regarding the patient- age, Troponin T level, gender, race, history of smoking, history of diabetes, history of hypertension, C-reactive protein level, history of high cholesterol and CK-MB level. After exclusion, the number of patient samples dropped to 523.

   These samples were measured for MPO levels, Troponin T levels, C-reactive protein levels, and CK-MB mass levels.

   Patients were followed for the development of major adverse cardiac events (MACE) over the next 6 months. Myocardial infarction was defined by Troponin T levels of at least 0.1 ng/mL. The need for revascularization was defined as coronary-artery bypass surgery, percutaneous coronary intervention or catheterization with stenosis in at least two major coronary vessels of more than 70%. The incidence of MACE was assessed by follow up phone calls at 30 days and 6 months.

   Differences between groups in the outcome and associations among categorical variables were assessed with the Wilcoxon rank-sum test. Trends for unadjusted analyses were evaluated with the Cochran-Armitage trend test. Correlations among continuous variables were assessed with the Spearman rank-correlation

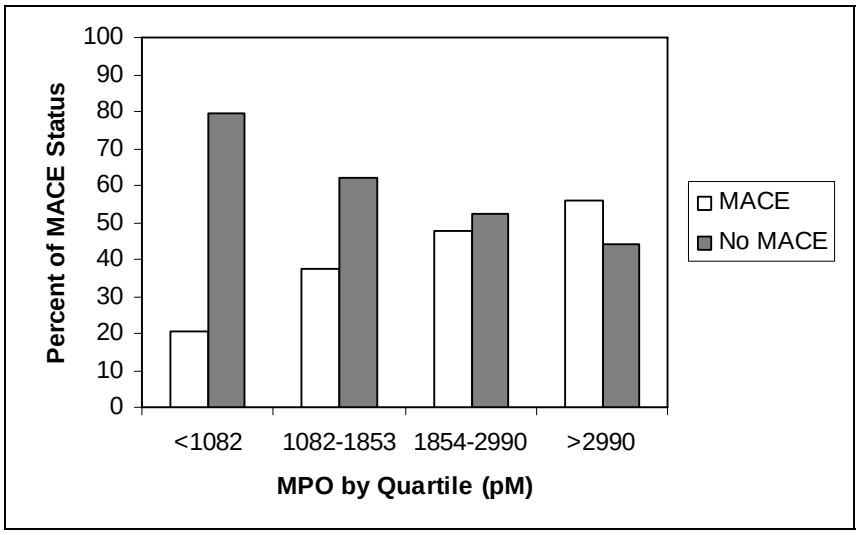
coefficient. Multivariate logistic-regression models (SAS version 8.0, SAS Institute) were developed to calculate odds ratios and 95% confidence intervals.

Plasma levels of MPO in patients presented with chest pain ranged from 181 to 10290 pM with a median of 1854 pM (25^{th} and 75^{th} percentile of 1082 and 2990 respectively). These levels differ significantly when compared to normal blood donors with plasma MPO levels with a median of 193 (25^{th} and 75^{th} percentile of 138 and 288 pM respectively). MPO levels in these patients weakly correlated with peak troponin T levels (r=0.22) and C-reactive protein levels (r=0.015) but not with age (r=0.068) or sex (r=0.081). MPO levels were significantly higher in current smokers, patients with a history of smoking, hyperlipidemia and revascularization in patients that had a MI within 16 hours after presentation. See the charted results below.

Characteristic	**Yes**	**No**	**P value**
	Median MPO level (n), 25^{th}-75^{th} % tile range		
Male sex	2017 pM (335), 1126-3240 pM	1699 pM (225), 1043-2843 pM	0.06
History of diabetes	2030 pM (143), 1157-3239 pM	1776 pM (402),1043-2897 pM	0.13
History of hypertension	1825 pM (358), 1055-3085 pM	1847 pM (188), 1111-2895 pM	0.97
History of myocardial infarct	1923 pM (196), 1176-3046 pM	1746 pM (337), 1024-2991 pM	0.21
History of coronary artery disease	1938 pM (258), 1160-3029 pM	1755 pM (272), 1009-2997 pM	0.19
Current smoking	2125 pM (130), 1180-3406 pM	1746 pM (401), 1029-2966 pM	0.03
History of smoking	2031 pM (332), 1103-3285 pM	1629 pM (201), 1043-2855 pM	0.03
History of hyperlipidemia	2138 pM (275), 1163-3406 pM	1652 pM (267), 1009-2800 pM	0.002
History of revascularization	2033 pM (184), 1237-3137 pM	1716 pM (350), 971-2991 pM	0.04
MI within 16 hours	2591 pM (128), 1546-3969 pM	1695 pM (432), 970-2797 pM	<0.001

The results of the MACE development over the next 6 months were summarized at 30 days and 6 months. Both of the time frames showed similar results. The 30 day results are shown below in table and graph form.

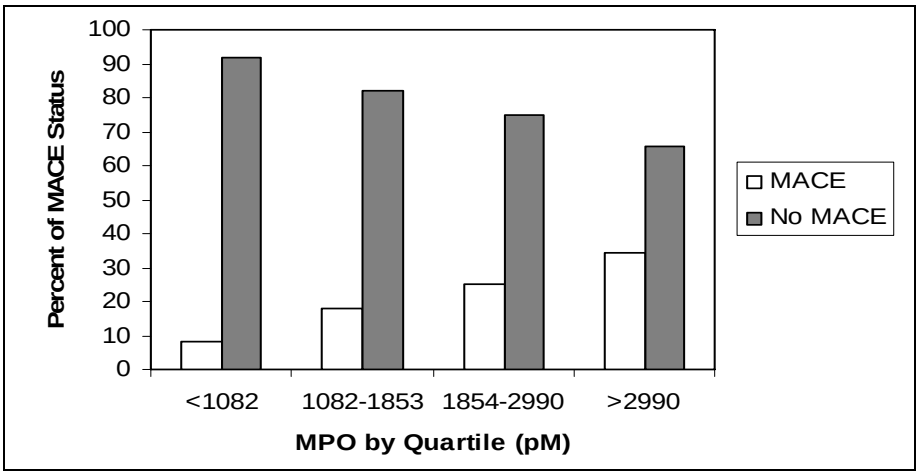
	MACE at 30 days, All Patients (n=523)			
MPO (pM)	**<1082**	**1082-1853**	**1854-2990**	**>2990**
Odds Ratio	1.0	2.5	2.8	3.3
95% CI	NA	1.3-4.8	1.5-5.6	1.7- 6.4
p value		p = 0.007	p = 0.0021	p < 0.001



The risk of MACE at 30 days increased with increasing MPO levels. An odds ratio analysis by the sponsor for MACE at 30 days by quartile of MPO level and revealed that increased levels of plasma MPO was a significant predictor of risk for MACE (3-fold increase for MPO in the 4th quartile).

The sponsor also conducted a similar analysis for the patients that were persistently negative for Troponin T which also revealed a significantly higher risk of MACE for patients with higher MPO levels. See table and graph below.

	MACE at 30 days, TnT negative Patients (n=304)			
MPO (pM)	**<1082**	**1082-1853**	**1854-2990**	**>2990**
Odds Ratio	1.0	3.4	4.1	6.9
95% CI	NA	1.2-9.4	1.5-11.4	2.5-19.2
p value		p = 0.0194	p =0.007	p < 0.001



The odds ratio for MACE at 30 days in patients without evidence of myonecrosis rises ~ 6.9 fold for those with MPO levels in the 4th quartile relative to those with MPO levels in the 1st quartile.

The sponsor claims that more patients who experienced MACE over the following six months were identified by the combination of either an elevated Troponin or MPO level.

4. Clinical cut-off**:**

   N/A

5. Expected values/Reference range:

   300 samples from apparently healthy subjects (150 of each sex) in the age range 22 to 60 (median age of 46) were evaluated. The sponsor states that there is no evidence of a correlation between MPO levels and age in the patients presenting with chest pain and felt it appropriate to analyze a population of "normal" subjects over an age range typical to patients with chest pain (40 years of age or more). The samples were analyzed in duplicates with the kit, controls and calibrators. A Box-Cox transformation was applied to all the data to fit a normal distribution and limits were set based on the 25th to 75th percentile range of the transformed data.

   The sponsor found no significant difference in MPO levels between sexes. The median MPO level for subjects in the adjusted normal subject population was 193 pM, the overall range of result was 48 to 924 pM and the interquartile range was 138 to 288 pM. The distribution is shown below. The sponsor states that 95% of the CardioMPO test results on a healthy normal population are expected to fall below 539 pM.

Percentile (%)	2.16	2.5	95	95.67	97.5	97.83
MPO (pM)	78	80	505	539	619	708

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.